Multiplex Reverse Transcription Polymerase Chain Reaction Assessment of Sialyltransferase Expression in Human Breast Cancer

Marie-Ange Recchi,1 Mohamed Hebbar,2 Louis Hornez, Anne Harduin-Lepers, Jean-Philippe Peyrat, and Philippe Delannoy3


Abstract

Increased sialylation, especially involving the Sialyl-LewisX and Sialyl-LewisX determinants, has been reported in breast cancer. A multiplex reverse transcription-PCR method was used here to determine the expression of five sialyltransferases (ST3Gal III, ST6Gal I, ST3Gal IV, ST3Gal I, and ST3Gal II) in 49 patients surgically treated for locoregional breast cancer. We assessed the relationship between these expressions and clinical, pathological, and biological features. The most expressed sialyltransferase was ST3Gal III, which is involved in Sialyl-LewisX synthesis. ST3Gal III expression was positively correlated to ST6Gal I and ST3Gal IV expressions, to tumor size, and to the number of involved axillary nodes. Patients with high ST3Gal III expression had a shorter overall survival. High ST6Gal I expression was associated with histopathological grade III. ST6Gal I expression was negatively correlated to expression of progesterone receptor. In conclusion, high ST3Gal III and ST6Gal I expressions in human breast tumors are associated with poor prognosis markers.

Introduction

Modifications of cellular glycosylation are a common phenotypic change in malignancy. However, only a limited number of biosynthetic pathways are frequently altered in cancer. Increased β1,6-branched, increased Sialyl-LewisX [NeuAcα2→3Galβ1→4(Fucα1→3)GlcNAc] and Sialyl-LewisX [NeuAcα2→3Galβ1→4(Fucα1→4)GlcNAc] epitopes, or the general increase in sialylation of cell surface glycoproteins are commonly observed in N-linked and O-linked oligosaccharides of carcinoma cells. These changes in glycosylation are related to grade, invasion, metastasis, and with a poor prognosis (1, 2).

Carbohydrate changes also occur in breast cancer. Sialyl-LewisX, Sialyl-LewisX, and Sialyl-Tn (NeuAcα2→6GalNAcα1→O-Ser/Thr) are tumor-associated antigens found in breast cancer (3–5). It has been shown that these epitopes are attached to, among other proteins, the MUC1 apoprotein (6). The CA 15–3 assay detects the circulating form of a high molecular weight sialomucin MUC1 and is a very powerful marker of advanced stages of breast cancer (7). Moreover, E-selectin, a cell surface adhesion molecule that interacts with Sialyl-LewisX and Sialyl-LewisX, is secreted as a soluble form (sE-selectin) in breast cancer sera. High concentrations of sE-selectin are associated with reduced overall survival (8).

The biosynthesis of sialylated oligosaccharide sequences are catalyzed by a family of enzymes named sialyltransferases. These enzymes use CMP-sialic acid as the activated sugar donor. More than 15 different sialyltransferases acting on glycoproteins and/or glycolipids have been characterized to date, and 8 different sialyltransferase cDNAs have been cloned from human tissues or cells (9).

Changes in sialyltransferase expression have been observed in cancer tissues or cells, and the regulation of their expression is achieved mainly at the transcriptional level. For example, transfection of rat fibroblasts with the ras oncogene leads to an increase of the β-galactoside α2,6-sialyltransferase (EC 2.4.99.1, ST6Gal I) mRNA, and of the invasion potential of these cells (10). Recently, Ito et al. (11) demonstrated by quantitative RT-PCR that in human colorectal cancer, the ST3Gal I mRNA was prominently increased in cancer tissues compared with nonmalignant colorectal mucosa.

Very little investigation into the expression of sialyltransferases in human breast cancer has been carried out. However, one study conducted on breast cancer cells showed an elevated activity in the transfer of sialic acid onto Galβ1→3GalNAc-R acceptor substrates (12). This elevated activity could explain the higher level of sialylation of MUC1 found in these cells (13).

Recently, we have developed a sensitive multiplex RT-PCR method (14) that can monitor the expression of four human sialyltransferases: ST6Gal I, ST3Gal I, ST3Gal III, and ST3Gal IV (15); these are involved in the biosynthesis of the sialylated structures of glycoproteins (Table 1). This method has been extended to a fifth enzyme recently cloned (16). The aim of the present study was to assess the expression of these five sialyltransferases in patients treated for loco-regional breast cancer, and to assess the relationship between this expression and the clinical, pathological, and biological features of these patients.

Materials and Methods

Patients and Cells. This retrospective study involved 49 unselected patients undergoing surgery for locoregional breast cancer in the Center Oscar Lambret (the Anticancer Center of the North of France, Lille, France). Patients were included between March 1990 and June 1993. The mean age of the patients was 61.1 ± 10.5 years (SD; range, 36–80). None of the patients had evidence of distant metastasis. In the studied population, the minimum and the median durations of follow-up of living patients were 9 months and 65 months, respectively. Within this population, the number of deaths was 22 (44.8%) and the number of relapses was 18 (36.7%). HepG2 cells from Dr. B. Laine (Institut Pasteur, Lille, France) were from American Type Culture Collection (HB 8065) and were cultured in DMEM supplemented with 10% FCS, 1% nonessential amino acids, 2 mM glutamine, and antibiotics. Cells were grown at 37°C in a humidified atmosphere of 5% CO2.

1 The nomenclature of sialyltransferases: ST3Gal I: CMP-NeuAc: Galβ1→3GalNAc α2,3-sialyltransferase, EC 2.4.99.4; ST3Gal II: CMP-NeuAc: Galβ1→3GalNAc α2,6-sialyltransferase, EC 2.4.99.1; ST6Gal I: CMP-NeuAc: Galβ1→4GlcNAc α2,3-sialyltransferase, EC 2.4.99.4; ST6Gal II: CMP-NeuAc: Galβ1→4GlcNAc α2,6-sialyltransferase, EC 2.4.99.1.

2 These authors have contributed equally to this work.

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**Table 1** Specificity of human sialyltransferases and sequences of the six primer pairs used for PCR amplification

<table>
<thead>
<tr>
<th>Target DNA</th>
<th>Acceptor specificity</th>
<th>Primers set</th>
<th>Guanosine and cytidine content (%)</th>
<th>Melting temperature (°C)</th>
<th>PCR product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH (J04038)</td>
<td>Galβ1-3(4)GlcNac</td>
<td>5′-<strong>TTGGGACCTAGCCCTGCTT</strong>-3′</td>
<td>63</td>
<td>73.1</td>
<td>256</td>
</tr>
<tr>
<td>ST3Gal III</td>
<td>Galβ1-3(4)GlcNac</td>
<td>5′-<strong>AGGGCCACACCCACTGTTG</strong>-3′</td>
<td>63</td>
<td>73.4</td>
<td>300</td>
</tr>
<tr>
<td>ST3Gal I</td>
<td>Galβ1-4GlcNac</td>
<td>5′-<strong>GAATCCAGGCTGGCTGA</strong>-3′</td>
<td>60</td>
<td>70.3</td>
<td>371</td>
</tr>
<tr>
<td>(X17247)</td>
<td>Galβ1-4GlcNac</td>
<td>5′-<strong>GAAGCTGGTTAGCTGGCAGT</strong>-3′</td>
<td>50</td>
<td>70.2</td>
<td></td>
</tr>
<tr>
<td>ST3Gal IV</td>
<td>Galβ1-4(3)GlcNac</td>
<td>5′-<strong>CAGGAAAGCTCATGACACCT</strong>-3′</td>
<td>50</td>
<td>69.1</td>
<td></td>
</tr>
<tr>
<td>ST3Gal III</td>
<td>Galβ1-3GlcNac</td>
<td>5′-<strong>CCCTATGTGGAGGGCTTGGG</strong>-3′</td>
<td>50</td>
<td>59.1</td>
<td></td>
</tr>
<tr>
<td>(L29555)</td>
<td>Galβ1-3GlcNac</td>
<td>5′-<strong>CCTGCTTTGGTCAGCTGTTG</strong>-3′</td>
<td>50</td>
<td>458</td>
<td></td>
</tr>
<tr>
<td>ST3Gal II</td>
<td>Galβ1-3GlcNac</td>
<td>5′-<strong>CTCTCGGCGAAGCAGCTG</strong>-3′</td>
<td>50</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>(U63090)</td>
<td>Galβ1-3GlcNac</td>
<td>5′-<strong>GAGGGCCACACCCACTGTTG</strong>-3′</td>
<td>50</td>
<td>71.2</td>
<td></td>
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<tr>
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<td>5′-<strong>GAGGGCCACACCCACTGTTG</strong>-3′</td>
<td>50</td>
<td>71.2</td>
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<td>50</td>
<td>71.2</td>
<td></td>
</tr>
</tbody>
</table>

*Genbank accession numbers are shown in brackets.
*Primer sequences are shown in the 5′ to 3′ orientation. Enzyme-related area: GAPDH-related area.

**Pathology.** After surgery, the tumor samples were divided into three parts:
two parts were frozen for hormone-receptor and sialyltransferase analyses, and the third part was submitted for histological examination. Tumor samples consisted solely of invasive adenocarcinomas. The HPG was obtained using the Scarff and Bloom’s criteria (17). Both ER and PgR were determined by the dextran-coated charcoal method, as described previously (18).

**sE-selectin Assay.** Serum samples were obtained from the patients before surgery. Blood (5 ml) were collected in EDTA, and were centrifuged at 3,000 × g for 10 min. Sera were stored at −20°C until analysis. Concentrations of sE-selectin were measured in duplicate using a commercial ELISA kit (R&D Systems, Inc., Abington, United Kingdom) as described previously (8). To avoid a possible rise in sE-selectin concentration associated with impaired liver or kidney functions, we verified that all patients had normal liver (serum bilirubin ≤ 30 μmol/l; aspartate transaminase ≤ 25 IU/ml) and renal (serum creatininine ≤ 130 μmol/l) function.

**Isolation of Total RNA.** Total RNA was isolated with the use of Tri-Reagent from Sigma Chemical Co. (St. Louis, MO). The tumor samples were homogenized in 1 ml of Tri-Reagent with a Potter homogenizer. Following the supplier’s recommendations, to minimize the possibility of DNA contamination when isolated RNA is to be used in RT-PCR, we performed three additional steps in the extraction procedure: (a) the homogenates were centrifuged at 12,000 × g for 10 min at 4°C to remove the insoluble material; (b) we proceeded with a phenol/chloroform extraction; and (c) the precipitation of RNA with isopropanol was performed in two steps. The RNA pellet was then washed with 750 μl of ethanol, centrifuged, and allowed to dry for 5-10 min by air-drying. The amount of the RNA was quantified by measuring the absorbance at 260 nm.

**RT-PCR.** Total cellular RNA (5 μg) was heated at 65°C for 10 min and placed on ice for 2 min. Reverse transcription into cDNA was achieved using the First-Strand cDNA Synthesis kit (Pharmacia Biotech), according to the manufacturer's protocol using oligo-d(T) as initiation primer in a final reaction volume of 33 μl. The retrotranscription reaction (4 μl) was subjected to PCR amplification (14), we never detected any unspecific amplification product; this demonstrates the high specificity of the primer pairs used for PCR amplification. Using serial dilutions of HepG2 cDNA as positive control, we demonstrated the linearity of the multiplex sialyltransferase amplification. After completion of the 1/6 of the GAPDH PCR reaction mixture or 1/6 of the sialyltransferase multiplex PCR reaction mixture were coelectrophoresed through a 6% polyacrylamide-8 M urea gel on a 373 DNA Sequencing System from Applied Biosystem (Perkin-Elmer Corp., Norwalk, CT). Molecular mass markers fluorescently labeled with 6-carboxy-X-rhodamine (Applied Biosystem) were also coelectrophoresed. The results were analyzed with Genescan 672 Software (Applied Biosystem). Levels of each sialyltransferase expression were presented as the ratio enzyme-related area:GAPDH-related area.

**Statistical Analyses.** A difference between the expression of the five sialyltransferases was searched using a nonparametric one-way ANOVA (Kruskall-Wallis test). A relationship between the sialyltransferase expression and other parameters was assessed using the χ² with Yates correction. A positive or negative correlation between the expression of each sialyltransferase was assessed using the Spearman nonparametric test. A linear correlation was also performed after log transformation. Overall survival and relapse-free survival curves were calculated using the Kaplan and Meier method. Comparison between curves was carried out by the log rank test. Graphic representations were performed using the box-plot method (19). In this representation, the box extends from the 25-75th percentile, with a horizontal line at the median. Whiskers extend down to the smallest value and up to the largest. Single points are considered outliers.

**Results**

**Clinical and Pathological Features.** Tumor size was ≤3 cm in 29 cases (59.1%) and >3 cm in 20 cases (40.8%) of the total 49 cases. An axillary nodal involvement was found in 28 of 49 patients (57.1%). HPG was obtained in 38 of 49 cases (77.5%). It was grade I in 2 cases (5.2%), grade II in 18 cases (47.3%), and grade III in 18 cases (47.3%) of the 38 cases analyzed. ER and PgR were obtained in all cases. A positivity for ER and PgR was found in 42 (85.7%) and 36 (73.4%) of the 49 cases analyzed, respectively. The mean concentration of circulating sE-selectin was 32.6 ng/ml ± 17.3 (SD; range, 9.7-111 ng/ml).

**Sialyltransferase Expression.** An individual example of the profiles obtained with biopsies is presented in Fig. 1a. Whereas the fluorescent multiplex RT-PCR technique is 50-fold more sensitive than the previously described ethidium bromide revelation (14), we never detected any unspecific amplification product; this demonstrates the high specificity of the primer pairs used for sialyltransferase amplification. Using serial dilutions of HepG2 cDNA as positive control, we demonstrated the linearity of the multiplex...
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Fig. 1. A, example of a profile of the fluorescent peak corresponding to the five sialyltransferases (full line) and GAPDH (dotted line). The GAPDH PCR reaction mixture (1/60) or the sialyltransferase multiplex PCR reaction mixture (1/6) were coelectrophoresed through a 6% polyacrylamide-8 M urea gel on a 373 DNA Sequencing System and analyzed by Genescan 672 Software (Applied Biosystem). Molecular mass markers fluorescently labeled are indicated (full urea). B, box-plot graphic representation of the relative expression of the five sialyltransferases in 49 human tumor biopsies. Levels of each sialyltransferase expression were presented as the ratio enzyme-related area:GAPDH-related area. The expression of the five sialyltransferases were compared using the Kruskal-Wallis lest. The expression of ST3Gal III was significantly higher than the expressions of ST3Gal IV and ST3Gal II (P < 0.05 and P < 10^{-3}, respectively), the expression of ST6Gal I was significantly higher than the expressions of ST3Gal IV and ST3Gal II (P < 0.05 and P < 10^{-3}, respectively), and the expressions of ST3Gal IV and ST3Gal I were significantly higher than the expression of ST3Gal II (P < 10^{-3} and P < 10^{-3}, respectively).

RT-PCR for the five sialyltransferases up to 40 PCR cycles and for the GAPDH amplifications, according to the area peak values obtained with the 5' fluorescently labeled primers (data not shown).

Absence of expression was found in 0, 1, 9, 3, and 39 cases for ST3Gal III, ST6Gal I, ST3Gal IV, ST3Gal I, and ST3Gal II, respectively. The median levels of ST3Gal III, ST6Gal I, and ST3Gal IV expressions were, respectively, 74.8 (range, 4.4–645.2), 63.9 (range, 0–370.6), and 24.5 (range, 0–85.9). The median expression of ST3Gal I was 69.2 (range, 0–436.1). ST3Gal II had a lower expression level (1.9; range, 0–42.0; Fig. 1B). This low level of expression was not due to the experimental procedure because this enzyme expression was always detected in HepG2, the positive control. Using the Kruskal-Wallis test, the expression of ST3Gal III was significantly higher than the expressions of ST3Gal IV and ST3Gal II (P < 10^{-3} and P < 10^{-3}, respectively), the expression of ST6Gal I was significantly higher than the expressions of ST3Gal IV and ST3Gal II (P < 0.05 and P < 10^{-3}, respectively), and the expressions of ST3Gal IV and ST3Gal I were significantly higher than the expression of ST3Gal II (P < 10^{-3} and P < 10^{-3}, respectively).

Correlations. Using the Spearman rank test, the expression of ST3Gal III was positively correlated to the expression of ST3Gal I (r = 0.46; P < 10^{-3}), the expression of ST3Gal II (r = 0.45; P = 0.001), and the expression of ST3Gal IV (r = 0.36; P = 0.01). The expression of ST6Gal I was correlated to the expression of ST3Gal I (r = 0.31; P = 0.03). The expression of ST3Gal IV was correlated to the expression of ST3Gal I (r = 0.46; P < 10^{-3}), and the expression of ST3Gal II (r = 0.45; P = 0.001).

Using the chi-squared test with Yates correction, the tumors with higher ST6Gal I expression (higher than the median value) was more frequently HPG III than the tumors with low ST6Gal I expression (13 of 19 versus 5 of 14; P = 0.009).

Using the Mann-Whitney test, patients with HPG III (n = 18) had significantly higher median ST6 Gal I expression than patients with HPG II (n = 18; P < 0.05).

Using the Spearman rank test, the expression of ST3Gal III was positively correlated to the tumor size (r = 0.31; P = 0.03) and to the number of involved axillary nodes (r = 0.32; P = 0.02). The expression of ST6Gal I was negatively correlated to the PgR expression level (r = -0.37; P = 0.009). No correlation was found between the expression of sialyltransferases and the expression of ER or the sE-selectin concentration.

For overall survival analyses, the patients with high ST3Gal III expression (higher than the median value) had significantly reduced survival (P = 0.043; Fig. 3). No significant difference was found for other sialyltransferases. Especially, the analysis of the Kaplan-Meier survival curves for ST6Gal I and ST3Gal I showed no significant difference when patients with expression lower than the median value were compared with those with expression higher than the median.
Discussion

In this study, we analyzed the tumoral expression of five sialyltransferases in 49 patients treated for locoregional breast cancer by using multiplex RT-PCR. This method allows a simultaneous assessment of all these enzymes in each tumor. We found that most tumors preferentially expressed the ST3Gal III. Moreover, the expression of ST3Gal III was correlated to the expression of three other enzymes (ST6Gal I, ST3Gal IV, and ST3Gal I). Interestingly, we found that ST3Gal II was weakly (or not) expressed in breast tumors. This enzyme shared the same substrate specificity with ST3Gal I. Both enzymes are able to transfer a sialic acid residue onto the 3-OH group of the Gal residue of the type III disaccharide structure (Galβ1-3GalNAc). Because of this common substrate specificity, both enzymes could be involved in the sialylation of T-antigen (Galβ1-3GalNAcα-O-Ser/Thr) currently observed in O-glycoproteins, especially in mucins. Both enzymes exhibit different tissue-specific expressions and our result indicates that the α2,3-sialylation of T-antigen in breast tumor would be essentially catalyzed by ST3Gal I.

The comparison between the expression of each sialyltransferase and standard prognostic factors showed that the highest expression of ST3Gal III was mainly found in large tumors, which suggests that this expression is not an early phenomenon. In agreement with this result, ST3Gal III expression was associated with axillary nodal involvement. Moreover, despite the relatively small size of the series for survival analyses, we found that a high expression of ST3Gal III was associated with a reduced overall survival. According to the substrate specificity of the sialyltransferases, ST3Gal III, which prefers type I disaccharidic acceptor, is the candidate for the synthesis of SiaL±Lewisx ligand and prognostic factors or tumoral characteristics.

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value. No more differences were found when other cutoff values were used (first and third quartiles). Finally, for relapse-free survival, no significant difference was found for any sialyltransferase.

In this study, we analyzed the expression of ST3Gal III and the expression of ST6Gal I in 49 human tumor biopsies. The distribution of the expression of ST3Gal III and ST6Gal I were log normal. The values none-zero were transformed to expression of SToGal I in 49 human tumor biopsies. The distribution of the expression of 3GlcNAc) or type II (Galβ1-4GlcNAc) disaccharide sequences; and a2,3-sialylation of the terminal galactose residue of type I (Galβ1-3GlcNAc) or type II (Galβ1-4GlcNAc) disaccharide sequences; and (b) the fucosylation of the GlcNAc residue, in α1–4 in the case of Sialyl-Lewisx, or in α1–3 in the case of Sialyl-LewisX. In such biosynthetic pathways involving α2–3-sialylation before fucosylation, the increased expression of ST3Gal III could lead to the increased expression of Sialyl-Lewisx. In that context, our result brings further evidence for the participation of the interaction between endothelial selectins and their tumoral counterparts in tumor development and progression. Because the concentration of circulating sE-selectin may be a marker of endothelial cell activation and a reflection of cell-surface E-selectin expression, we compared these concentrations with the tumoral expression of sialyltransferases. We didn't find any correlation that can support this hypothesis. A study performed in a larger series may be useful to determine whether patients with both an important endothelial E-selectin expression (reflected by the sE-selectin concentration) and an important tumoral ST3Gal III expression have a worse outcome.

ST6Gal I expression was also associated with bad prognosis indicators. We observed a positive correlation between ST6Gal I expression and both the grade III HPG, and the lack of hormone receptor expression. ST6Gal I was previously shown to be regulated by steroid hormones at the transcriptional level: ST6Gal I mRNA level is increased in rat hepatocytes and in rat fibroblasts by dexamethasone, leading in both cases to an increased number of NeuAcα2–6Galβ1–4GlcNAc sequences at the cell surface (for review see Ref. 9). On the other hand, the progressive loss of sialic acids of the brush-border membrane glycoproteins (one of the major biochemical changes occurring in rat small intestine during the transition from suckling to weaning) is associated with a decreased expression of ST6Gal I mRNA, which is speeded up by injection of hydrocortisone (20). At this stage, we cannot assume that the elevated expression of ST6Gal I is directly associated with the lack of steroid hormone receptors, but this question should be addressed in future experiments. Nevertheless, the expression of ST6Gal I mRNA has been previously associated with malignant transformation. Increased expression of ST6Gal I mRNA has been described in cultured cells transformed by oncogene transfection (10), and the elevated expression of ST6Gal I mRNA in adenocarcinomatous human colon compared with normal tissues has been recently reported (21). In our study, this enzyme is correlated with a poor prognosis, suggesting an important role of ST6Gal I in malignant transformation, and a study performed in a larger series may show a prognostic value of ST6Gal I expression in patients with breast cancer.

In the present study, no clear link could be established between the expression of ST3Gal IV (the enzyme involved in the synthesis of the Sialyl-Lewisx ligand) and prognostic factors or tumoral characteris-

Fig. 2. Spearman rank test correlation between the expression of ST3Gal III and the expression of ST6Gal I in 49 human tumor biopsies. The distribution of the expression of ST3Gal III and ST6Gal I were log normal. The values none-zero were transformed to logarithms, and a linear regression analysis was performed \[ R^2 = 0.41, p < 10^{-4}, y = 0.65X + 0.52. \]
tics. However, previous studies have indicated an increased expression of Sialyl-LewisX in breast cancer. Renkonen et al. (22) indicate that epithelial expression levels of Sialyl-LewisX are even higher in metastatic tumors compared with primary lesions. Type II disaccharidic sequences (Galβ1-4GlcNAcβ1-R) are the precursors of Sialyl-LewisX epitopes, but can be used as acceptor substrates by different other glycosyltransferases (i.e., the β-galactoside α2,6-sialyltransferase, ST6Gal I, the α1–2-fucosyltransferase leading to Lewisα antigen), and the over expression of Sialyl-LewisX epitopes could also reflect a change in the expression of this competitor enzyme. Nevertheless, in the present study in breast cancer tissues, ST3Gal IV expression is correlated with the expression of ST3Gal III, which synthesizes preferentially Sialyl-LewisX. In another model, the study by Ito et al. (11) indicates the presence in the same amount of these two enzymes in colorectal cancer tissues compared with nonmalignant colorectal mucosa. In the same way, by the analysis of >300,000 transcripts derived from at least 45,000 different genes, Zhang et al. (23) have identified >500 transcripts that were expressed at significantly different levels in normal and neoplastic gastrointestinal cells. Within these transcripts, ST3Gal IV was one of the largest decreases in expression in tumor cells, whereas the expression of ST3Gal III is not affected. These results suggest that if the expression of sialyltransferases is involved in the prognosis of cancer grade or survival, it depends on the type of cancer.

Finally, Sialyl Tn expression is correlated in breast cancer with prognostic parameters (5). The amount of ST6GalNAc I sialyltransferase message, which synthesizes this motif, remains unknown because this enzyme is not already cloned from human source.

In conclusion, the multiplex RT-PCR, which can provide the analysis of the expression of five sialyltransferases in biopsies in one reaction, showed the correlation of the expression of ST3Gal III and ST6Gal I with poor prognosis factors in patients with breast cancer.

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References

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